Unusual Case of Suspected Acute Porphyrria

To the Editor:

The presentation of a darkly stained urine specimen must, in addition to other diagnostic possibilities, raise the suspicion of an acute porphyrinic attack. High urinary excretion of the porphyrin precursors, porphobilinogen (PBG) and α-levulinic acid, is the major biochemical abnormality observed in three porphyrrias: acute intermittent porphyrria (AIP), variegate porphyrria (VP), and hereditary coproporphyria (HCP).

AIP, although rare, is the commonest of the acute porphyrrias. The initial symptom is usually lower abdominal pain, which is often colicky and may last from hours to days. Other symptoms include pain and parasthesia in the extremities, nausea, vomiting, constipation, and back pain (see ref. 1 for review). In remission, urinary PBG excretion decreases but rarely to within reference intervals.

Excess amounts of PBG can be rapidly detected in freshly voided urine by a qualitative screening test: the modified Watson–Schwartz test (2), which has commonly been used as a first line of investigation in cases of suspected acute porphyrria. The method does, however, yield both false-negative (3, 4) and false-positive results, and a positive result should be confirmed by quantitative measurement of PBG. We recently encountered a case that emphasizes the problems associated with the modified Watson–Schwartz test. Indeed, this test is no longer used in our laboratory, having been replaced by a quantitative chromatographic method for PBG assay (Bio-Rad, Munich, Germany).

A 14-year-old boy presented at the Accident and Emergency (A&E) Department with suspected hematuria. On arrival he went into the lavatory on his own and produced a urine specimen that looked dark red. He was started on antibiotics for a suspected urinary tract infection. However, subsequent laboratory investigations on this urine sample showed no evidence of red or white blood cells, and microbiological culture and measurements of protein and glucose were negative. Hepatic and renal function tests and hematological investigations all gave results that were within reference limits.

Several days later, the patient's physician requested a urinary porphyrin screen after observing "a bucket full of red urine" at the patient's home and suspecting, in the absence of hematuria, an acute porphyrin. A urine sample received in the dark at the laboratory at this time was colorless and no increased concentrations of porphyrins or PBG were detected. After a further episode of severe back pain and darkly colored urine, the boy presented once more at the A&E Department, at which time a bright red urine sample was sent to the Clinical Chemistry Department for analysis. Urine culture of this sample gave no growth, as did a test for the presence of hemoglobin.

In view of the history of back pain and the prescription of an antibiotic considered "unsafe" in patients with AIP, an acute porphyrin appeared to offer a possible explanation for the colored urine. The sample received from the A&E Department, although not kept in the dark, was qualitatively screened for the presence of PBG with the modified Watson–Schwartz test (2), and gave a strongly positive result. A control test, in which Ehrlich's reagent was replaced by 7 mol/L hydrochloric acid, also showed red coloration of the lower layer after extraction, indicating the presence of dye. The patient's physician was then contacted to obtain yet another fresh urine sample in the dark for quantitative investigation. When this sample arrived at the laboratory, it was colorless, and the modified Watson–Schwartz test gave a negative result for PBG.

Because the grossly high concentrations of an acute porphyrin attack persist for months and commonly years (2), the absence of PBG in a urine sample collected only 48 h after a supposed attack raised strong suspicions of a tampered sample. The pediatric consultant was informed and met with the patient and his parents. Faced with the laboratory evidence, the boy admitted coloring the urine with beetroot (beet) juice, and the case was referred to a child psychiatrist. Psychiatric evaluation of this patient concluded that there was no obvious psychiatric pathology and, rather than being a case of Munchausen syndrome, it was felt to be an example of hysterical behavior. No further action was taken.

There have been several documented cases of factitious hematuria in children and adolescents (5, 6); this case is unusual in that the substance added to the urine was not blood but a colored agent that the patient presumably believed would be mistaken for blood. Consumption of quantities of beetroot is a recognized cause of red urine, but this is the first case recorded at this laboratory in which beetroot juice was directly added to a urine sample.

In laboratories where the modified Watson–Schwartz test is used as a first-line investigation, this case emphasizes the importance of including a control test, in which red color in the lower layer after extraction indicates the presence of indicator dyes that occasionally appear in urine after ingestion of coloring agents in medicine, sweets, soft drinks, and various foodstuffs, or in colored substances added directly to the urine as demonstrated here. The inclusion of a chloroform extraction step, as originally described by Watson and Schwartz (7), will detect false-positive results that are due to exogenous foodstuffs.

We thank the staff of the Clinical Chemistry Department at the Central Pathology Laboratory and the Department of Child Psychiatry of the North Staffordshire Hospital.

References
Comparing Assays of Antibodies to Modified Low-Density Lipoproteins

To the Editor:

A recent surge of interest in the role of autoimmune processes in the pathogenesis of atherosclerosis has resulted from convergent work on low-density lipoproteins (LDL) by several different groups, which established that LDL-anti-LDL immune complexes induce foam cell formation, that oxidized LDL (ox-LDL) is immunogenic and can be detected in atheromatous lesions, and that antibodies to ox-LDL can be detected in the peripheral blood of patients with atherosclerosis and healthy controls, as well as in atheromatous lesions (recently reviewed in ref. 1).

The methodology described by Craig et al. in this journal (2) raises some important questions concerning the specificity of the assays used by most investigators in the field. With slight variations, most published assays of antibodies to different types of modified LDL are based on determining the difference or the ratio between the binding of a given sample to modified LDL and to native LDL (2-4). However, we believe that this approach leads to erroneous values, because it ignores the fact that modification of LDL adds negative charge to the LDL molecule (5), increasing the potential for charge-dependent noncovalent interactions with IgG.

The possible interference of these nonspecific interactions is aggravated by the fact that the antibodies to ox-LDL appear to be of low affinity. We developed an affinity chromatography technique to isolate anti-ox-LDL antibodies, using immobilized ox-LDL prepared by the CuCl₂ technique (6) as substrate. Unbound proteins were eluted at very low ionic strength (0.01 mol/L Na₂CO₃ buffer), and bound anti-ox-LDL antibodies were eluted with 0.1 mol/L Na₂CO₃ buffer + 0.5 mol/L NaCl. These conditions have allowed us to isolate antibodies from six different individuals, and we have found that anti-ox-LDL antibodies cross-react with malondialdehyde-modified LDL (MDA-LDL). In addition, we were able to calculate the average dissociation constant of one of the purified anti-ox-LDL antibodies, which was $8 \times 10^{-7}$ mol/L, compared with an average dissociation constant of $7 \times 10^{-6}$ mol/L for rabbit anti-human LDL antibodies.

Consequently, we prefer to use a competitive assay to quantify anti-ox-LDL antibodies (7), in which the difference in binding to ox-LDL of two aliquots of each sample—one absorbed with the same ox-LDL preparation used to coat the enzyme immunosassay (EIA) plates, the other diluted with phosphate-buffered saline (PBS)—is considered to reflect the antibody concentration. We decided to compare the two approaches to the determination of anti-MDA-LDL and anti-ox-LDL antibodies in 20 random samples, part of an ongoing study of anti-ox-LDL antibodies. The competitive assay, previously described by us (7), was adapted to the assay of anti-MDA-LDL antibodies by using MDA-LDL prepared as described by Haberland et al. (8) to coat the wells of EIA plates and to absorb the antibody. The differential assay involved determining the different reactivity of identical dilutions of each serum against native and modified LDL. To minimize variation, we tested the aliquots against the two types of lipoprotein in question in the same plate, of which one-half was coated with modified LDL and the other half with native LDL. The LDL concentration used to coat EIA plate wells was constant in all assays (0.75 mg/L). Other constant factors were blocking solution (bovine serum albumin, 50 g/L in PBS) and the conjugated antibody [5000-fold-diluted peroxidase-conjugated IgG fraction of rabbit anti-human IgG (cat. no. 55221; Cappel/Organon Teknika Corp., Durham, NC)]. The results were expressed as the differences in absorbance (414 nm) between unabsorbed and absorbed samples for the competitive assays, and as the differences in absorbance between readings obtained with modified LDL and native LDL for the differential assay. All values were calculated from triplicates, two samples run at one dilution and a third at a higher dilution. Because plates coated with MDA-LDL absorb significantly more IgG than plates coated with ox-LDL, we diluted samples 10- and 20-fold for the assays of anti-ox-LDL and 100- and 200-fold for the assays of anti-MDA-LDL.

We found that the competitive assay yielded higher values for anti-ox-LDL than for anti-MDA-LDL, whereas the differential assay yielded consistently higher values for anti-MDA-LDL. Fig. 1 illustrates the comparisons between the competitive assays for anti-ox-LDL and anti-MDA-LDL and between the competitive and differential assays for anti-MDA-LDL. There was basically no correlation between the results of the two assays for anti-MDA-LDL ($r = 0.153$ by linear regression, statistically not significant). A comparison between competitive and differential assays for anti-ox-LDL antibodies (not illustrated) yielded $r = 0.3966$ (also not significant). In contrast, the results of